REMARKS

This submission is in response to the Official Action dated June 4, 2001. Claims 107-125, 130-131, and 133-136, have been canceled, without prejudice or disclaimer. New claims 146-169 have been added. The Examiner has made the restriction requirement dated October 4, 2000, final. Accordingly, claims 1-106, 126-129, 132, and 136-169 are pending, and claims 146-169 are at issue. Reconsideration of the above identified application, in view of the above amendments and the following remarks, is respectfully requested.

The specification has been amended to identify each sequence discussed in the specification with the corresponding SEQ ID NO. Specifically, the figure text for Figure 22 (page 13, line 29) has been amended to identify both the nucleotide (SEQ ID NO:14) and amino acid (SEQ ID NO:15) sequences for the pelB signal peptide. In addition, the sections discussing the HRP1A6 protein (page 13, lines 30-31; page 60, lines 4-5) have been amended to identify the nucleotide (SEQ ID NO:16) and protein (SEQ ID NO:17) sequences, and the sections describing primers on page 59, lines 1-2 have been amended to identify the corresponding sequence identifiers; SEQ ID NOS: 18 and 19.

Moreover, the Sequence Listing filed April 30, 1999, has been replaced with a Substitute Sequence Listing. The Substitute Sequence Listing identifies that SEQ ID NOS: 1 and 2 are *P. Putida* sequences (supported by the specification at page 29, lines 23-27); that SEQ ID NOS: 3-10 are primer sequences (see Examples); and

that SEQ ID NOS: 11-13 are mutants (supported by the specification at page 55, lines 21-28). In addition, the bibliographic information of the Sequence Listing has been corrected to list all priority applications. A statement under 37 C.F.R 1.821 is provided below.

Claims 115-122, 124-125, and 133-136 have been canceled in favor of new claims 146-160. Claims 107-114 and 130-131 have been canceled in favor of new claims 161-169. New claims 146-169 are supported by the specification as filed. This is shown in the following table, where original claims and sections in the specification which support each new claim are exemplified.

New Claim	Original Claim	Specification
146	115	page 55, lines 21-28
147	116	page 55, lines 21-28
148	117	page 55, lines 21-28
149	118	page 55, lines 21-28
150	119	page 55, lines 21-28
151	120	page 55, lines 21-28
152	121	page 55, lines 21-28
153	122	page 55, lines 21-28
154	124	page 55, lines 21-28; p. 21, lines 1-15
155	125	page 55, lines 21-28; p. 21, l. 32 to page 32, l. 12
156	133	page 3, lines 1-3
157	134	page 3, lines 1-3
158	135	page 15, lines 11-18

159	136	page 15, lines 11-18
160		page 55, lines 21-28
161	107, 1, 134	page 3, lines 1-3; page 27, lines 1-28
162	20	page 24, lines 7-12
163	47, 20	page 14, lines 9-16; page 16, lines 3-23
164	107, 1, 136	page 27, lines 1-28, page 3, lines 1-3
165	20	page 24, lines 7-12
166	47, 20	page 14, lines 9-16; page 16, lines 3-23
167	107, 1, 121	page 27, lines 1-28, page 55, lines 21-28
168	20	page 24, lines 7-12
169	47, 20	page 14, lines 9-16; page 16, lines 3-23

No new matter has been added by this amendment. Each objection and rejection in the Office Action is discussed below, using the same section numbers as the Office Action, and to the extent that the same objections and rejections may be relevant to the claims replacing the claims canceled with this response.

Oath/Declaration

The Examiner considers the Declaration defective because it does not state the U.S. provisional application numbers upon which the priority dates have been claimed.

4. Applicants respectfully submits that this objection is in error. There is no requirement by Rule 1.67(a), (b), or (c) that the Declaration must state any priority

claimed from U.S. provisional applications (only foreign applications), and Applicants are not aware of any other Rule which sets forth such a requirement. Hence, this objection should be withdrawn.

Drawings

5. In response to the objections to the drawings, Applicants have enclosed formal drawings which obviate these objections.

Claim Objections

6. The Examiner has objected to claims 107-114 and 130-131 for depending from non-elected claims. Claims 107-114 and 130-131 have been canceled with this amendment, without prejudice or disclaimer. This objection is therefore obviated.

THE REJECTIONS UNDER 35 U.S.C. §112, 2nd PARAGRAPH, SHOULD BE WITHDRAWN

8-9. The Examiner has rejected claims 115-122, 130-131, 133-136 as allegedly indefinite for either reciting the short form "P450 oxygenase" instead of "cytochrome P450 oxygenase" or for reciting "P40 oxygenase."

With this response, all claims directed to a cytochrome P450 oxygenase recite "cytochrome P450 oxygenase."

10. The Examiner has rejected claim 122 as allegedly indefinite for reciting three mutations at the same amino acid position.

New claim 153, which essentially corresponds to former claim 122, recites mutations at positions 331, 280, and 242.

11. The Examiner has rejected claim 125 for reciting "a first polynucleotide" and a "second polypeptide".

New claim 155, which essentially corresponds to former claim 125, recites "a first polynucleotide" and "a second polynucleotide".

12. The Examiner has rejected claim 130 for reciting the phrase "a provided P450 enzyme".

In the new claims added with this amendment, this terminology is no longer used.

13. The Examiner has rejected claims 115-125, 130-131, and 133-136, as allegedly indefinite for not providing a wild-type enzyme sequence against which the variant enzyme characteristics can be compared.

New claims 147-158 all call for variant enzymes which have mutations at positions corresponding to the recited positions of cytochrome $P450_{cam}$ from *P. Putida* (SEQ ID NO:2).

Accordingly, in view of the above amendments and remarks, all rejections under 35 U.S.C., 2nd paragraph, should be withdrawn.

THE REJECTIONS UNDER 35 U.S.C. §112, 1ST PARAGRAPH, SHOULD BE WITHDRAWN

14. The Examiner has rejected claims 107-125, 130-131, and 133-136 as allegedly not being enabled by the specification. Specifically, the Examiner contends that the specification enables a mutant cytochrome P450 oxygenase isolated from *P. Putida* having specified mutations, but not any mutant/variant cytochrome P450 oxygenase enzyme from any source. The Examiner also alleges that the specification does not establish (A) a rational and predictable scheme for isolating and characterizing any variant cytochrome P450 oxygenase from any given source with an expectation of obtaining the desired activity and function; (B) the modification tolerance of such enzymes, and (C) guidance as to which of the choices is likely to be successful.

The pending claims call for variant enzymes which have mutations at positions corresponding to the recited positions of cytochrome $P450_{cam}$ from *P. Putida* (SEQ ID NO:2), functional variants of such enzymes, variant enzymes encoded by polynucleotides which hybridize to polynucleotides encoding such enzymes, or variant cytochrome P450 enzymes that exhibit a higher stability or oxygenase activity than wild-type cytochrome P450 from *P. Putida*. These embodiments of the invention are all enabled by the specification.

"To be enabling, the specification of the patent must teach those skilled in the art how to make and use the full scope of the claims of invention without 'undue experimentation.' " Genentech Inc. v. Novo Nordisk, A/S, 42 USPQ 2d 1001, 1004 (Fed. Cir. 1997) (quoting In re Wright, 27 USPQ 2d 1510, 1513 (Fed. Cir. 1993)). The court has held that a patent specification complies with a statute even if a "reasonable" amount of routine experimentation is required but such experimentation must not be "undue". "[A]n extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance." *In re Colianni*, 561 F.2d 220, 224, 195 USPQ 150, 153 (CCPA 1977). " ' The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.'" *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404, (Fed. Cir. 1988) (citing In Re Angstadt, 537 F.2d 489, 502-04, 190 USPQ 214, 217-19, (CCPA 1976)).

Contrary to the Examiner's contentions, the specification describes methods for isolation and purification of enzymes (page 21, lines 16-31), and both such methods and sources for oxygenase enzymes are well-known in the art (specification, page 5, line 16 to page 7, line 17). Moreover, the specification, in particular the Examples, provides ample guidance on methods for creating and characterizing variant enzymes. In fact, the method of the invention set forth in, *e.g.*, claims 160-169, and described in detail in Examples 1-10 of the specification, is in

itself a method for characterizing an enzyme. This method is suitable for, *e.g.*, determining modification tolerance and whether an enzyme would be successful by estimating the oxygenase activity or stability of a test enzyme. Thus, although experimentation to identify the mutants of the invention might require an extended period of experimentation, such experimentation would not be undue, since the specification as a whole provides both the guidance and direction necessary, particularly in the sections referred to above.

Specifically, for new claims 146-152 and 167-169, the amino acid sequence for a variant cytochrome P450 enzyme, prepared as described in the Examples, can be aligned with SEQ ID NO:2 using a suitable sequence alignment algorithm (see specification, page 21, lines 7-11) to identify which residue corresponds to the positions of the specific mutations set forth in the claims. For new claims 153 to 155, a function conservative variant can be identified as described at page 21, lines 1-15 of the specification, and a hybridizable polynucleotide identified as described by the specification at page 21, line 32 to page 22, line 12. For new claims 156-159 and 161-166, a cytochrome P450 variant of the invention can be searched for and identified using the screening method discussed above. While such an analysis might be laborious if a large number of mutants are screened, such experimentation would not be "undue" according to the definition of the courts.

Accordingly, the invention as set forth by the claims is enabled, and this rejection should be withdrawn.

15. The Examiner has rejected claims 107-125, 130-131, and 133-136 as allegedly not meeting the written description requirement. The Examiner alleges that no information beyond the characterization of a single variant with amino acid substitutions at positions 331, 242, and 280 of wild-type cytochrome P450 oxygenase has been provided which could indicate possession of the claimed genus of modified enzymes, and that there are many structurally and functionally unrelated polypeptides encompassed within the scope of the claims.

The enzymes of the claimed invention all have the same unifying function; oxygenase activity, *i.e.*, they are all enzymes which promote the addition of oxygen to a substrate. This function is described in the specification (*e.g.*, at page 15, line 19 to page 16, line 2), and methods for assaying the same are described throughout the specification and in the claims. Particularly the Examples describe methods for high-throughput screening of multiple oxygenase enzymes. Further defining features of the invention include the description of enzymes variants or evolved enzymes (p. 20, ll. 25-30; p. 25, l. 29 to p. 26, l. 2; p. 8, l. 1 to p. 9, l. 15).

Moreover, cytochrome P450 oxygenases is a particularly well-defined group with known structural and functional features (p. 5, l. 25 to p. 7, l. 29). New claims 146-159 correlate the structural and functional features of the variant cytochrome P450 enzymes of the invention with those of cytochrome P450 oxygenase from *P. putida*, providing a reference point against which the enzymes genus of the invention is clearly defined. As described in the specification (*e.g.*, at

page 21, lines 7-15), and discussed above, a variant cytochrome P450 enzyme sequence could be aligned with the corresponding wild-type sequence (for a *P. Putida* cytochrome P450 variant; SEQ ID NO:2) using an algorithm such as MEGALIGN to identify the positions corresponding to the positions set forth by the claims.

Thus, Applicants respectfully requests reconsideration and withdrawal of this rejection.

THE REJECTIONS UNDER 35 U.S.C. §102 SHOULD BE WITHDRAWN

The Examiner has rejected claims 107-114, 123-125, 130-131, and 133-136 as being anticipated by either one of Manchester et al. (Protein Eng. 1995;8:801-807; hereinafter "Manchester 1995") or Manchester et al. (Biochemie 1996;78:714-722; hereinafter "Manchester 1996"). In the Examiner's opinion, the Manchester references disclose variant cytochrome P450 oxygenases that can be identified using a method comprising the steps set forth in the claims. The Manchester 1995 reference also discloses a variant cytochrome $P450_{cam}$ (F87W) that dechlorinates pentachloroethane approximately three times faster than the wild-type enzyme (abstract). Since both Manchester 1995 and 1996 stand or fall on the same premises, Applicant addresses them jointly as "Manchester".

New claims 146-155, 160 and 167-169 call for mutations or amino acid sequences not taught or even suggested by Manchester. Likewise, new claims 157-

159, and 161-166 directly or indirectly call for variant enzymes which are either characterized in having a stability at least 2 or 10 times higher than wild-type cytochrome P450 from *P. putida*, or characterized in having an oxygenation activity at least 2 or 10 times higher than wild-type cytochrome P450 oxygenase from *P. Putida*. Manchester is only concerned with ***dehalogenation*** properties of a single cytochrome P450 mutant which has three times higher dechlorination activity than the parent enzyme, and nothing is taught or suggested regarding enzyme stability or enzyme activity in promoting the oxygenation of a substrate in these references. In fact, whether Manchester's mutant has ***any*** oxygenation activity is unknown, since the dehalogenation reaction investigated by Manchester only can take place in the complete absence of oxygen (Manchester 1995, 2nd column, 1st paragraph). Dehalogenase activity is ***not*** the same as oxygenase activity. In summary, Manchester does not disclose any of the specific cytochrome P450 mutations set forth in claims 146-155 160, or 167-169; or any cytochrome P450 enzyme having the at least 2 or 10 times higher stability called for by claims 158-159 and 164-166; or any cytochrome P450 enzyme having at least 2 or 10 times higher oxygenation activity than wild-type cytochrome P450. Since a reference cited in a proper anticipation reference must disclose each and every element of the claims in question, Manchester does not anticipate the instant invention. Consequently, this rejection should be withdrawn.

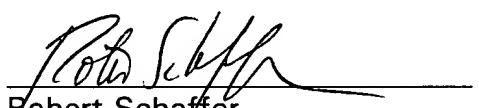
STATEMENT PURSUANT TO 37 C.F.R. § 1.821

Enclosed herewith is a substitute paper copy and substitute computer readable form (diskette) containing sequence disclosures. Pursuant to 37 C.F.R. § 1.821, Applicants hereby confirm that the contents of the paper copy of the substitute Sequence Listing filed herewith and entitled "SEQUENCE LISTING", and of the identically labeled diskette enclosed herewith, specifically the ASCII-encoded file therein labeled "Seqlist.txt", are identical. This sequence submission contains no new matter.

Therefore, in view of the above amendments and remarks, it is respectfully requested that the application be reconsidered and that all pending claims be allowed and the case passed to issue.

If there are any other issues remaining which the Examiner believes could be resolved through either a Supplemental Response or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

Respectfully submitted,



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PATENT TRADEMARK OFFICE

Docket No: 9373/1E827US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Frances H. ARNOLD; Hyun JOO

Serial No.: 09/246,451 Art Unit: 1652

Filed: February 9, 1999 Examiner: M. Rao

For: OXYGENASE ENZYMES AND SCREENING METHOD

MARK-UP FOR AMENDMENT

Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

November 5, 2001

Sir:

IN THE SPECIFICATION:

Please amend the specification pursuant to 37 C.F.R. 1.121 as follows.

Deleted subject matter is indicated by double brackets: "[" and "]".

Amend the paragraph on page 13, line 29, to read as follows:

FIG. 22 shows the coding sequence of the pelB signal peptide ([SEQ. ID. NO. 14 and 15]).

Amend the paragraph on page 13, lines 30-31, to read as follows:

FIG. 23 shows a nucleotide and amino acid sequence encoding an HRP enzyme variant designated HRP1A6 ([SEQ. ID. NO. [15] 16 and SEQ. ID. NO. [16] 17]).

Amend the paragraph on page 59, line 1, to read as follows:

5'-TTATTGCTCAGCGGTGGCAGCAGC [SEQ ID NO: [15] 18], and

Amend the paragraph on page 59, line 1, to read as follows:

5'-AAGCGCTCATGAGCCCGAAGTGGC [SEQ ID NO: [16] 19].

Amend the paragraph on page 60, lines 1-5, to read as follows:

Sequencing of the mutant gene found a mutation at position 255, in which the codon AAC for the amino acid asparagine (Asn or N) was changed to the codon GAC for the amino acid aspartic acid (Asp or D). This residue is a putative glycosylation site, and is located at the surface of the protein. The sequence of this mutant (HRP1A6) is shown in FIG. 23 [SEQ. ID NO. [15] 17]. A map of a plasmid pETpelBHRP1A6 containing this mutant is shown in FIG. 24.